Journal of Experimental Botany, Vol. 48, No. 317, pp. 2037–2043, December 1997

Journal of Experimental Botany

Structure of potato tubers formed during spaceflight

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Received 18 March 1997; Accepted 16 July 1997

Abstract

Potato (*Solanum tuberosum* L. cv. Norland) explants, consisting of a leaf, axillary bud, and small stem segment, were used as a model system to study the influence of spaceflight on the formation of sessile tubers from axillary buds. The explants were flown on the space shuttle Columbia (STS-73, 20 October to 5 November 1995) in the ASTROCULTURE® flight package, which provided a controlled environment for plant growth. Light and scanning electron microscopy were used to compare the precisely ordered tissues of tubers formed on Earth with those formed during spaceflight. The structure of tubers produced during spaceflight was similar to that of tubers produced in a control experiment. The size and shape of tubers, the geometry of tuber tissues, and the distribution of starch grains and proteinaceous crystals were comparable in tubers formed in both environments. The shape, surface texture, and size range of starch grains from both environments were similar, but a greater percentage of smaller starch grains formed in spaceflight than on Earth. Since explant leaves must be of given developmental age before tubers form, instructions regarding the regular shape and ordered tissue geometry of tubers may have been provided in the presence of gravity. Regardless of when the signalling occurred, gravity was not required to produce a tuber of typical structure.

Key words: Spaceflight, development, potato tuber, microgravity.

Introduction

Biological organisms have evolved under changing atmospheric and temperature conditions, but in a constant gravitational field. Thus, an organism's perception of and response to gravity are likely to be highly conserved. Gravity elicits characteristic growth patterns in the roots and shoots of plants (Sack, 1991), and force applied to plant cells grown in culture aligns the division plane of new cells (Lintilhac and Vesecky, 1984). Therefore, the absence of gravity may affect the form of entirely new plant structures (flowers produced by meristems developed in hypogravity) and of existing structures that undergo development (tubers that develop from lateral buds). Growth and development in a hypogravity environment are not as well-known, in part because research opportunities are limited and flight duration may be too short for new structures to form or develop.

To study the effects of spaceflight on processes associated with potato tuber formation, an experiment using explants was flown on the space shuttle Columbia (STS-73) in flight hardware capable of providing controlled environmental conditions (Morrow *et al.*, 1995). Potato explants, consisting of a leaf, its axillary bud, and a small stem segment, form sessile tubers of 1–2 g fresh weight in 14–16 d when placed in suitable conditions (Wheeler, 1986). Such explants are a model system for study of the physiological and developmental processes that occur during tuber formation (Wheeler, 1986). The tuber develops through a progression of cell divisions and cell expansion in the axillary bud. Axillary buds of leaves on intact plants expand as branches, duplicating the main bud in producing a leafy stem, whereas tubers form as

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storage organs, providing carbohydrates suitable for human consumption or serving as propagules that form entirely new plants.

It was hypothesized that without gravity profound changes in tuber development might occur. First, if tubers formed at all, they might be abnormal in shape since the axillary buds undergo precisely ordered cell divisions to produce the tuber tissues, and gravity might provide a cue for orienting cell walls. Second, starch grains might be less numerous or altered in form or composition because decreases in starch levels occur when plants are grown in conditions simulating spaceflight (Brown and Piastuch, 1994) and changes in starch grain size are known from previous spaceflights (Volkmann and Sievers, 1990).

Materials and methods

Potato explant system

Solanum tuberosum (cv. Norland) mother plants were grown from *in vitro* propagated plantlets, beginning six weeks before launch. Plants were grown at 21 °C, 80% RH, 150 μmol m^{-2} s^{-1} *PPFD* for a 12 h photoperiod and approximately 350 μmol mol^{-1} CO_2 in a walk-in growth chamber at the Kennedy Space Center. Flight explants were harvested from the 7th and 8th leaves of the mother plants, counting basipetally from the youngest leaf that was 1 cm in length. Each explant consisted of a leaf, axillary bud, and small section of stem. Since more leaf tissue was present than could be fit in the plant chamber of the flight hardware, the lamina was trimmed to fit by cutting off leaflet tips (Fig. 1). The stem and lower petiole region of each explant were placed into a tray filled with moistened arcillite, a calcined clay medium.

The above protocol also was used in baseline studies and in the control study performed at Madison. Explants in the control study, identical in developmental age to the flight explants, were harvested from mother plants grown at Kennedy Space Center and transported to Madison. When the ground control experiment was performed, the ASTROCULTURE® growth unit was programmed to operate as during spaceflight and placed in a controlled environment room at the UW-Madison Biotron to approximate the temperature, relative humidity, and CO_2 level surrounding the unit in the shuttle mid-deck during the flight.

ASTROCULTURE® growth unit

The ASTROCULTURE® unit, which was developed at the Wisconsin Center for Space and Automation Robotics, provides a controlled environment for growing plants in microgravity. The flight package was programmed to maintain the plant chamber at 21 °C, 80% RH, and a minimum of 500 ppm CO_2. Above the plant chamber was an array of red and blue light-emitting diodes (LEDs) to provide 150 μmol m^{-2} s^{-1} *PPFD* during the 12 h light period. Water was delivered to the arcillite-containing plant tray by a negative pressure system (Duffie *et al.*, 1995). These conditions were established as optimal for uniform tuber formation in ground-based studies using prototypes of the equipment; other flight hardware details can be found in Brown *et al.* (1996)..

Microscopy procedures

Before the mission, baseline studies were conducted on ground control explants of several ages by light microscopy (LM) and scanning electron microscopy (SEM). Hand sections of leaf, petiole, stem, and tuber tissue were examined by bright-field and fluorescence microscopy. Sections, some stained with I-KI and Coomassie Blue, were examined with a Zeiss Axioplan microscope and photographed using Kodak 2415 or Ektachrome 400 film. Tuber starch grains were collected on cover slips by abrading the cut surface of a tuber with a knife blade. Cover slips were attached to SEM stubs with glue, coated with 10 nm of gold-palladium, and examined at 10 kV with an Hitachi Model S-570 Scanning Electron Microscope. Images were recorded on Polaroid PN 55 film.

Measurements

Using an image analysis program (Cue 2, Olympus Corp.), starch grain length (longest axis) was determined from SEM photographs of whole granules (magnification 300 ×) collected from space and ground control tubers. More than 800 measurements of grains were made for tubers formed in space and on earth. Aspect ratios of starch grains were calculated by measuring the long axis and the widest short axis of each grain. The short axis measurement was divided by the long axis measurement to calculate the ratio.

Light microscope images were captured at 10 × and 40 × with a JVC TK-1070U colour video camera attached to an Olympus BH2 microscope, imported and printed using Photoshop 3.0 (Adobe Inc.). The number of cells in a standard area (49 cm^2 on the printed image) was counted and then individual cells were measured in two areas: at the periphery of the tuber, beneath the periderm, and at the tuber centre. Aspect ratios of these cells also were measured according to the same protocol used for starch grains.

Results

Leaf and petiole structure at harvest

Laminar tissue in the potato explants was dark green at the time of harvest from the mother plant (Fig. 1), and the intensity of autofluorescence in leaf transections showed that a large amount of chlorophyll was present (Fig. 2A). Plastids in the leaf mesophyll were starch-laden as indicated by IK-I staining (Fig. 2B). The petiole surface autofluoresced when irradiated with blue-violet light, an indication of a cuticular layer (Fig. 3), while no fluorescence was present at the leaf surface, indicating lack of a cuticle or presence of only a thin cuticle (compare Fig. 2A with Fig. 3).

Axillary bud structure at harvest

When explants were harvested from the mother plants, the axillary buds were approximately 1 mm in length. In spite of their small size, these buds had numerous leaf primordia and well-developed vascular tissue extending into the stem (Fig. 4A). In sectional view, the lateral buds consisted of prominent longitudinal files of cells, with pith cells that were greater in length than width and cortical cells that were nearly equal in length and width

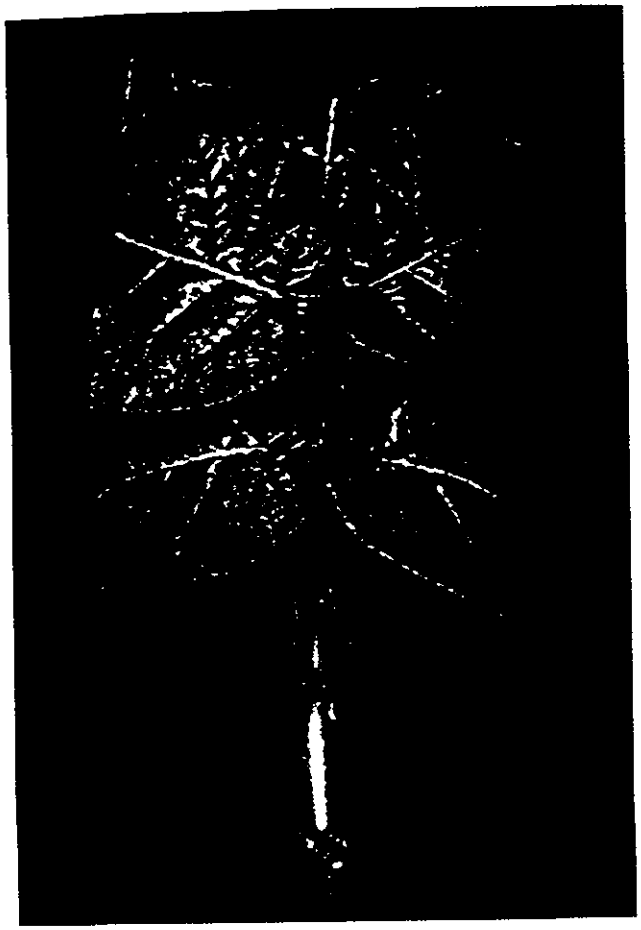

Fig. 1. Potato explant trimmed of leaflet tips to fit in the plant chamber of the ASTROCULTURE™ flight hardware. The axillary bud that develops into the tuber is not visible, but lies at the junction of the petiole and stem.

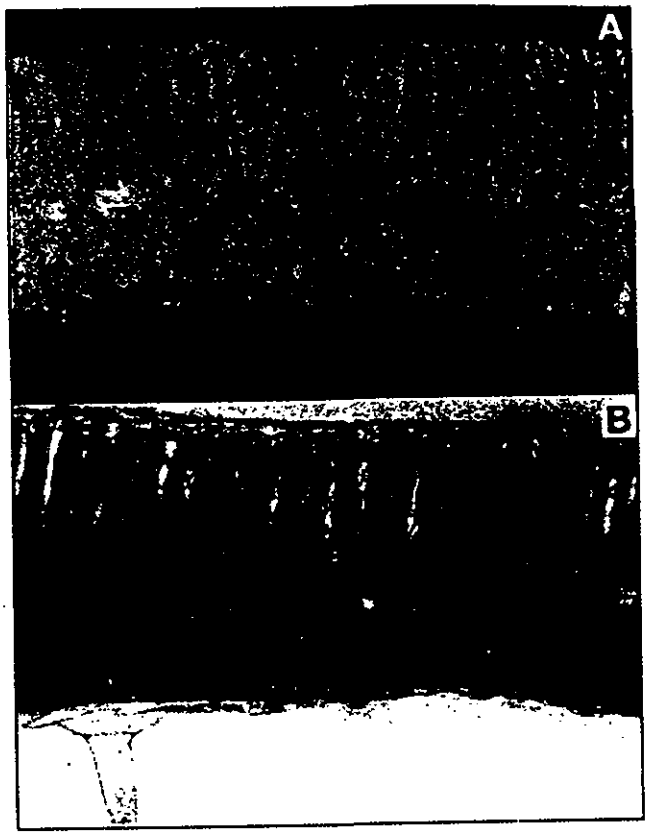

Fig. 2. (A, B) Lamina transection at explant harvest (baseline study) viewed by fluorescence (A) and brightfield (B) microscopy. In blue-violet light the chlorophyll in plastids of the palisade and spongy parenchyma cells autofluoresces red (A). The modest amount of yellow fluorescence present at the interface of the palisade and spongy parenchyma cells is vascular tissue. When the same section is stained with I-KI, the presence of starch is indicated by dark blue-black staining (B).

(Fig. 4A). Cells at the bud surface were tabular in shape and much smaller in overall dimensions (Fig. 4B). Because of the logistics of space shuttle experiments, the explants were harvested 36 h before lift off. Hence, learning whether buds began to develop on the ground before launch was essential. Buds collected 2 d after harvest exhibited no differences in cell dimensions, cell expansion, or file configuration (Fig. 4C) compared with buds collected immediately after harvest (Fig. 4B). In axillary buds of both ages, chlorophyll was present in leaf cells and anthocyanin was localized in the epidermis of all shoot meristems (Fig. 4A, C). Starch was detected in interior bud cells (data not shown).

Structure of tubers formed during spaceflight and on the ground

After 16 d of development the tubers produced in space and on the ground were comparable in shape (Fig. 5A, B) and weight (Tibbitts *et al.*, 1996). Since the tubers were nearly spherical, diameter rather than volume was measured. The tubers formed were similar in diameter

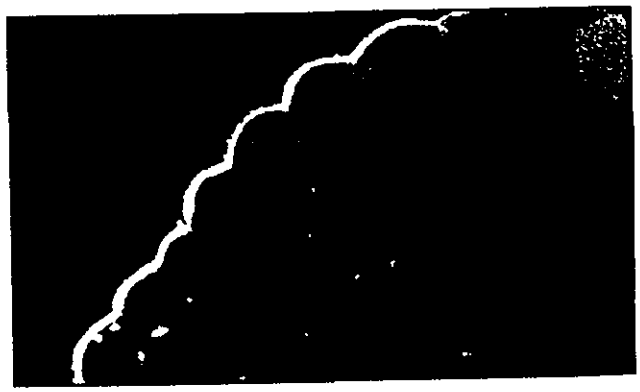

Fig. 3. Petiole sector in transection (baseline study) viewed by fluorescence microscopy (excitation filter 395–440 nm), emission filter, LP 470 nm). A band of yellow-green fluorescence indicating the cuticular layer is present at the surface above the large epidermal cells. Collenchyma is present at the interior and identifiable by wall thickenings at junctions of cell facets

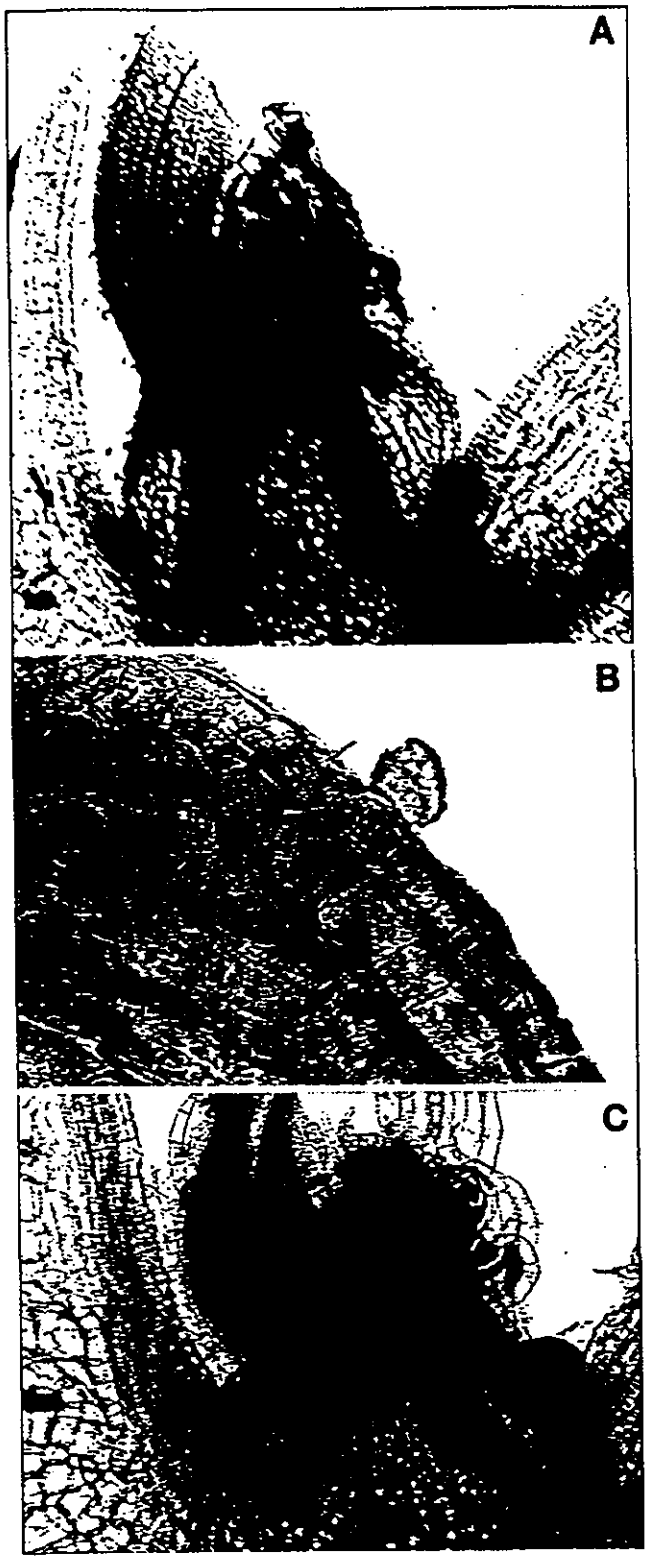

Fig. 4. (A–C) Longitudinal sections of axillary buds at harvest (A, B) and 48 h later (C); magnification of (A) and (B) is the same. At harvest (A) the axillary shoot bears multiple leaves and leaf primordia and shows developed vascular tissue. The edge of the stem (B) shows the difference in size and shape of cortical and epidermal cells. The epidermis is uniseriate at this stage. Forty-eight hours after harvest (C), the axillary bud is similar to the bud at the time of harvest from the mother plant.

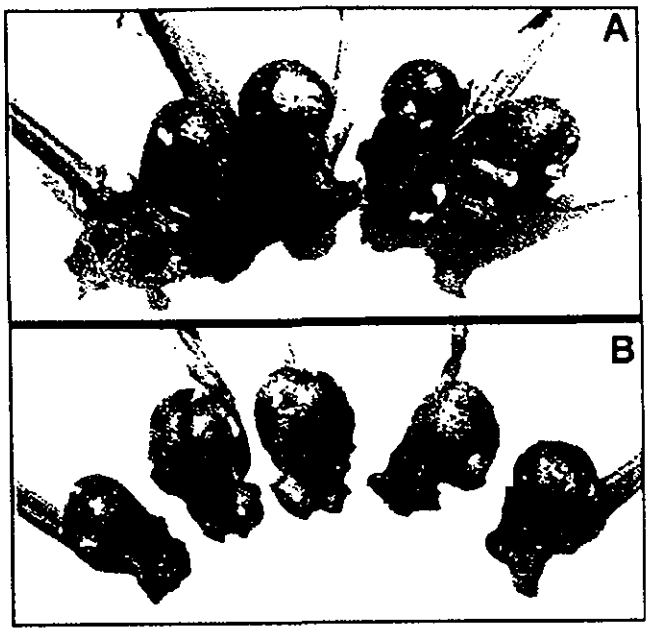

Fig. 5. (A, B) Potato tubers formed by explants during 16 d spaceflight (A) and ground (B) experiments. The tubers are comparable in size and shape.

(1.5 ± 0.8 cm, space; 1.5 ± 0.1 cm, ground) and weight (1.69 ± 0.34 g, space; 1.51 ± 0.28 g, ground). The organization of tuber cells was markedly altered from their arrangement in the axillary buds, but in agreement with previous studies of tuber structure (Cutter, 1992). The tuber tissues were organized in geometric arrays, with interior cells arranged in a honeycomb pattern (Fig. 6) and the surface cells ordered into a multilayered tissue (Fig. 7), with cells of a layer stacked directly on top of those of the previous layer. The number of layers in the

Fig. 6. Interior tuber cells from a baseline study showing their hexagonal arrangement.

Fig. 7. Unstained transection of the tuber periderm from a baseline experiment viewed by brightfield microscopy.

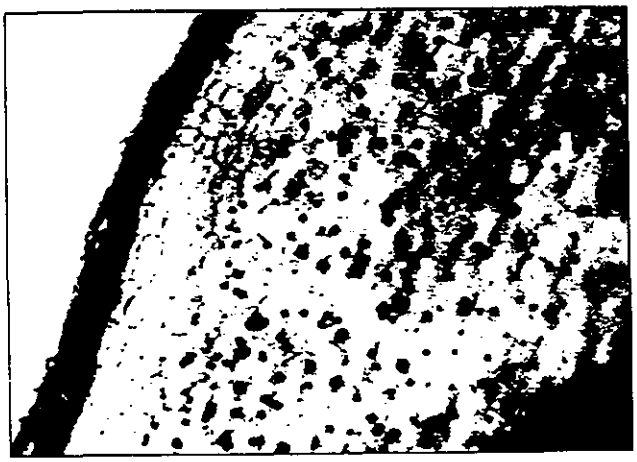

Fig. 8. A slice of tuber formed during spaceflight showing the multilayered periderm covering the interior cells arranged in a honeycomb. The dark inclusions in cells beneath the periderm are proteinaceous crystals that stain with Coomassie blue. These same interior cells also contain starch.

periderm was the same in flight (Fig. 8) and ground control tubers, and neither starch nor protein was detected in this tissue.

Measurements of cell area in sectional view just interior to the periderm and in the centre of tubers formed in space were comparable to cell measurements in equivalent regions of tubers formed in gravity ($605.53 \pm 224.26\ \mu m^2$, periphery space:$744.34 \pm 318.36\ \mu m^2$, periphery ground: $824.92 \pm 203.67\ \mu m^2$, interior space; $906.08 \pm 204.16\ \mu m^2$, interior ground; t-tests were not significantly different). Cell aspect ratios for comparable regions of space and ground tubers also were similar.

Starch and protein were found in the interior cells in ground control and spaceflight tubers (Fig. 8). Starch grains from tubers formed in flight (Fig. 9A) were comparable in character to those from ground control tubers (Fig. 9B). Starch grains ranged in shape from spherical to eccentric, with the smaller grains being spherical

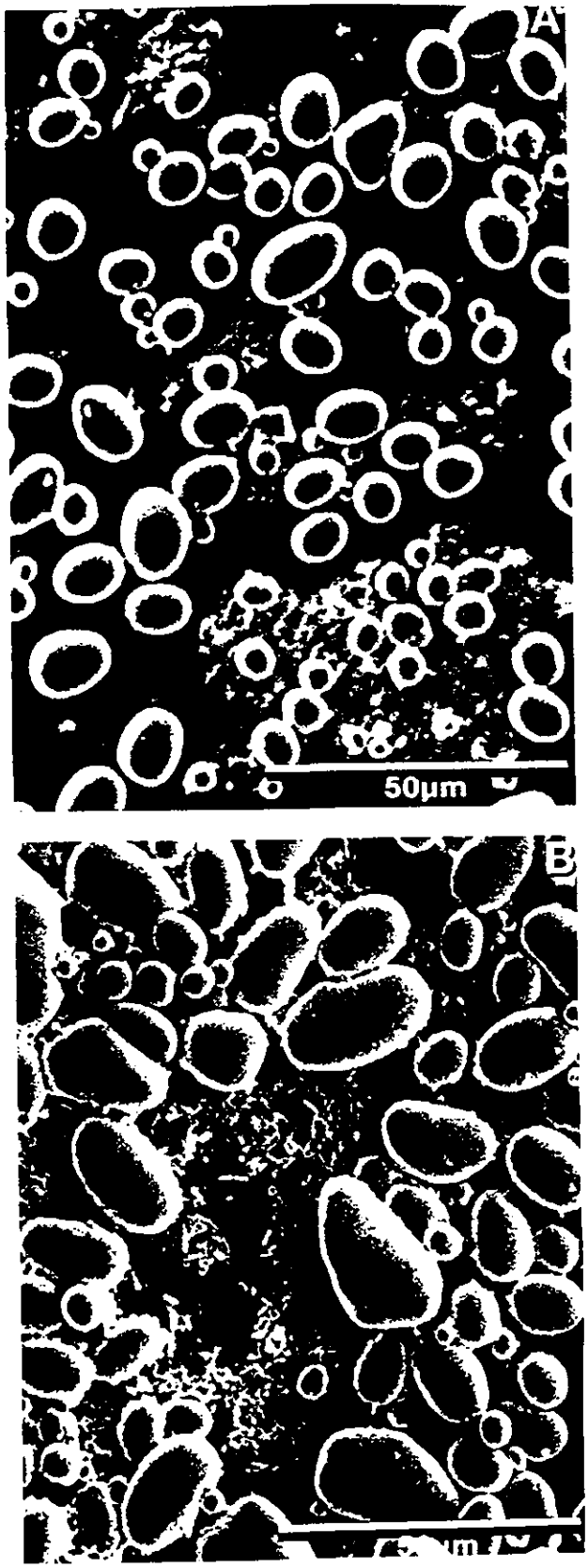

Fig. 9. (A, B) SEM images of starch granules from tubers formed in spaceflight (A) and ground (B) experiments. Bar equals 50 μm.

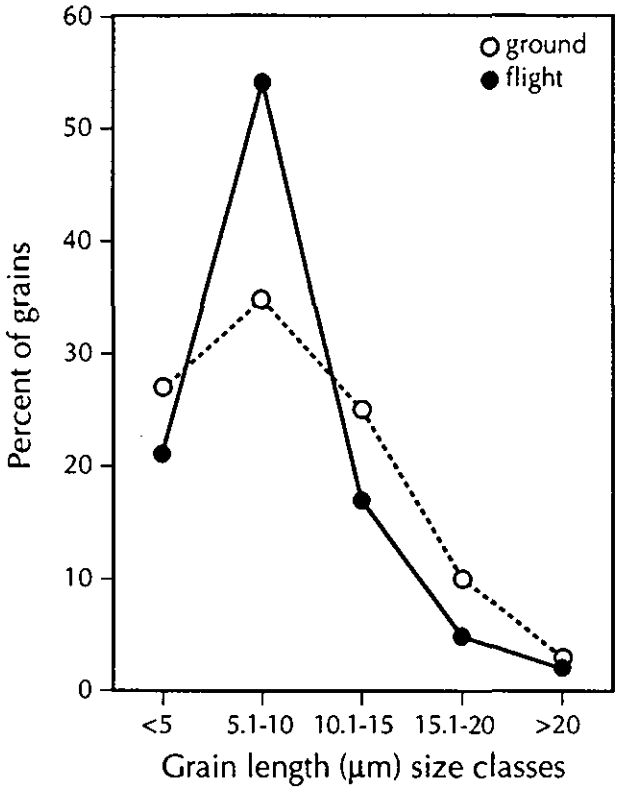

Fig. 10. Percentage of starch grains from spaceflight and ground tubers distributed into size classes by grain length.

(Fig. 9A, B). The surface of the grains was smooth. Grains ranged in size from 2–40 μm measured in the axis of greatest dimension. However, the distribution of grains into size categories differed between tubers formed in space and on the ground. More grains from spaceflight tubers were found in the smaller size classes than were found in earth-grown tubers (Fig. 10). The aspect ratio (length/width) of the grains formed in spaceflight was not different from that of control tuber grains.

Cells at the outer edge of the honeycomb region also contained crystals that stained with Coomassie blue, indicating that they were proteinaceous (Fig. 8). No obvious differences in protein size nor distribution within the tuber were evident.

Discussion

Gravity has been a steady and unchanging force during evolution. Given that organisms have evolved under this influence, sensing and responding to gravity are likely to be highly conserved mechanisms in all biological organisms. Since plants have specific gravitropic and gravimorphic responses (Sack, 1991), the absence of gravity may have profound effects on plant form. Interestingly, the effect of spaceflight on plant structure is thought to be modest (Claassen and Spooner, 1994). In fact, *Arabidopsis* produces flowers with functional reproductive organs and completes the sexual phase of its life cycle in space (Kuang *et al.*, 1996). However, evaluating the effects of spaceflight is complicated when cell origination and subsequent growth take place in different gravitational environments.

Results of spaceflight experiments with plants have been variable (Halstead and Dutcher, 1987; Claassen and Spooner, 1994), in part because of unplanned events that occur on particular missions, and because of differences in mission length, in experimental design, and in experimental equipment. Root growth provides an example of the complexity involved in analysing the effects of spaceflight. The growth of roots in length is sometimes comparable in spaceflight and control tissue, an observation made for more than a decade on many spaceflight missions using different orbiters and different flight hardware (Halstead and Dutcher, 1987; Claassen and Spooner, 1994). However, studies using a variety of genera show that the mitotic index declines during spaceflight, or upon return to a 1 *g* environment, and the number of dividing cells in the root apical meristem decreases because of premature differentiation. Root length does not differ from the control because of increased cell elongation (Krikorian and O'Connor, 1982; Merkys *et al.*, 1983; Merkys, 1990; Merkys and Laurinavičcius, 1990; Grif *et al.*, 1988, Darbelley *et al.*, 1989). Thus, morphological similarity may mask subtle changes in the mitotic cycle, cell differentiation, and cell expansion.

The potato explant system is advantageous for understanding effects of spaceflight because the tissue configurations in the tuber and in the axillary bud from which the tuber develops are highly ordered, each with its own characteristic arrangement of cells. When compared with tubers produced in a ground experiment, tubers formed in space were similar in size, shape, and weight. The similarity extended to internal tissues, where the tissue geometry of the periderm and of the interior cells was the same as control tubers, indicating that precise and regular patterns of cell division can occur during spaceflight.

Although the mitotic index of root meristem cells reportedly declines during spaceflight or after return to gravity (Halstead and Dutcher, 1987; Claassen and Spooner, 1994), no evidence was found that cell division or the rate of mitosis declined during tuber formation. The number of cell layers in the periderm and the number of cells/unit area in the tuber interior were the same as equivalent measurements in control tubers. Although no count of total tuber cells was made, similarity in tuber size and weight and cell area in sectional view was evidence that the number of divisions or the mitotic index was not altered in the microgravity of spaceflight.

The number and volume of organelles reportedly

change as a result of spaceflight (Volkmann and Sievers, 1990; Moore *et al.*, 1986), but in potato large differences in protein crystals and starch grains were not found in tubers in the two gravitational environments. Proteinaceous crystals were differentially distributed in flight tubers as they were in control tubers, with most large crystals found in cells beneath the periderm. No obvious difference in crystal number was apparent.

Since starch concentration declines in simulated microgravity (Brown and Piastuch, 1994), the similarity in the starch granules formed in space compared with controls was unexpected. The size, shape, and surface texture of starch grains formed in flight were comparable to those formed on earth. The only difference between starch grains formed in different gravitational environments was at the population level, i.e. their distribution into size classes. More small-sized grains formed in flight tubers compared with those formed on the ground. Since starch grain size increases with cell age in potato (Badenhuizen, 1968), the numerous small grains might indicate that cell maturity is delayed in space. Alternatively, the size of potato starch grains might have declined with mission length as has been found in roots (Moore *et al.*, 1986).

Microgravity has been reported to affect cell division, the mitotic cycle, cell differentiation and cell expansion. However, no evidence of such influence was found in the sessile tubers formed on potato explants during spaceflight. Although the ability to form a tuber from an axillary bud is developmentally regulated (Gregory, 1956), the presence of gravity was not required during tuber development using potato explants. If gravity acts as a developmental cue in tuber formation and structure in this model system, it probably acted while the leaves were present on the mother plant. Since the structure of the tubers formed in spaceflight showed no alterations, developmental instructions conferred in gravity were faithfully executed in microgravity.

Acknowledgements

The authors thank the crew and mission support staff of STS-73 in the successful performance of these experiments. Special thanks are due Dr Greg Goins and Dr Neil Yorio for the culture of the potato mother plants. We acknowledge NASA's Life and Biomedical Applications Division (Code UL) and NASA's Space Processing Division (Code XP) for financial support of this experiment. The authors also thank Claudia Lipke for photography and Kandis Elliot for assistance in the production of images. This research was supported by NASA grant NAGW 4022.

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